

## Phytochemical Screening of *Artocarpusaltilis* (Parkinson) Fosberg Unripe Fruit Extracts

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### Abstract

*Artocarpusaltilis*(Parkinson) Fosbergcommonly known as breadfruit belonging to *Moraceae* family is a monoecious tree widely grown in the hot, moist regions throughout southeast Asia, South India and most Pacific Ocean islands.Breadfruit has a wide range of application in traditional medicine andis primarily used as a food source. *Artocarpus* extracts and metabolites from leaves, stem, fruit, and bark contain numerous beneficial biologically active compounds and these compounds are used for various biological activities including antibacterial, antiviral, antifungal, antitubercularetc.In the present study five different cold percolation extracts of *Artocarpusaltilis*unripe fruit viz: aqueous, methanol, ethyl acetate, hexane, and acetone were screened individually to test the presence of various phytochemicals using standard procedures.Methanol extract showed the presence of nineteen phytochemical constituents followed by water, acetone, hexane and ethyl acetate.

**Key words :***Artocarpusaltilis*, solvents, cold percolation, phytochemicals.

### 1. Introduction

*Artocarpusaltilis* is one of the highest food yielding plants, with a single tree producing upto 200 or more grape fruit-sized fruits from March to June and from July to September (Akanbiet *al.*, 2009) per season and require limited care.As it is similar to freshly baked bread it is commonly called as

breadfruit. Breadfruit includes hundreds of varieties and thousands of common names which vary according to its geographic distribution, and is cultivated in over 90 countries (Morton and Julia, 1987). Breadfruit is also known as a traditional starch rich crop. Synonyms of *Artocarpus altilis* are *Artocarpus communis* and *Artocarpus incisus* (Orwa et al., 2014). Breadfruit grows in coral sands and saline soils. Over 130 compounds are identified in various organs of the tree of *Artocarpus altilis*, more than 70 of which derived from the phenylpropanoid pathway. The fruits contain artocarpine and the enzyme papayotine. The leaves contain the phenols, quercetin and camphorol, plus gamma-aminobutyric acid, which lowers blood pressure. The roasted flowers are rubbed on the gums around aching teeth to ease pain. Latex from this plant is bandaged on the spine to relieve sciatica. Diluted latex is also taken internally to treat diarrhea, stomach ache and dysentery. The mature yellow leaves have a capacity to reduce blood pressure levels and to control diabetes when brewed with tea. The root extract is an astringent and is also used as a purgative. Bark extracts had proven to possess strong cytotoxic activities against leukemia cells in tissue culture. Several studies had emphasized the antioxidant and antimicrobial potentiality of *Artocarpus altilis* along with its therapeutic properties as well as its effect on angiotensin-converting enzyme (ACE) activity. The ability of breadfruit pulp extract to inhibit cervical cancer cell proliferation was also demonstrated. The present study would emphasize the phytochemical strength of breadfruit.

## 2. Materials and Methods

### 2.1 Collection and processing of plant material

Mature unripe seedless fresh fruits of *A. altilis* (Parkinson) Fosberg were collected from Kanyakumari district, Tamil Nadu and brought to the laboratory, washed thoroughly, air dried in shade and reduced to coarse powder in a mixer grinder. The plant was authenticated by Dr. S. Mutheeswaran, Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India.

### 2.2 Preparation of unripe fruit extracts of *A. altilis* (Parkinson) Fosberg

Mature unripe seedless fresh fruits of *A. altilis* (Parkinson) Fosberg were sequentially extracted with different organic solvents such as hexane, ethyl acetate, acetone, methanol and water in increasing polarity (Starmans & Nijhuis, 1996). The crude extracts obtained were evaporated under reduced pressure at 40°C and finally concentrated and stored in a refrigerator at 2-8°C for use in subsequent experiments.

### 2.3 Phytochemical analysis of unripe fruit extracts of *A. altilis* (Parkinson) Fosberg

The solvent extracts of *Artocarpus altilis* fruits were subjected to phytochemical studies to screen the presence of phytoconstituents such as carbohydrates, quinones, alkaloids, amino acids, proteins, reducing sugars, flavonoids, gums and mucilages, tannins, resins,

terpenoids, phenols, saponins, cardiac glycosides, volatile oils, starch, betacyanins, coumarins, steroids, anthraquinone, phlobatannins, emodols and fattyacids .

### **2.3.1 Molisch's test for carbohydrates (Sofowora, 1993)**

The extracts were treated with 2 drops of alcoholic alpha-napthol solution in a test tube and 2 ml concentrated  $H_2SO_4$  was added carefully along the sides of the test tubes. Formation of red ring at the interphase indicated the presence of carbohydrates.

### **2.3.2 Test for quinones ( Evans, 1996 )**

To 1 ml of each solvent extract 1 ml of concentrated  $H_2SO_4$  was added . Formation of red colour would indicate the presence of quinones .

### **2.3.3 Test for alkaloids (Evans, 1997)**

The extracts were treated with Mayer's reagent ( 1.36 gm mercuric chloride and 5 gm of potassium iodide was dissolved in 100 ml distilled water ). The formation of yellow cream predicted the presence of alkaloids.

### **2.3.4 Ninhydrin test for aminoacids( Yasuma and Ichikawa, 2000 )**

To each of the solvent extract 0.25% ninhydrin reagent was added and boiled for few minutes for the development of blue colour .

### **2.3.5 Test for proteins**

The extracts were treated with 1 ml of 10% NaOH solution and heated . To this extract a drop of 0.7%  $CuSO_4$  solution was added. Formation of purplish violet colour was observed.

### **2.3.6 Benedict's test for reducing sugars (Tiwari *et al.*, 2011 )**

The extracts were treated with Benedict's reagent and heated on a water bath. Orange red coloured precipitate was formed.

### **2.3.7 Ferric chloride test for flavonoids (Raman, 2006 )**

The extracts were treated with few drops of  $FeCl_3$  solution . Formation of a blackish red

colour indicated the presence of flavonoids .

### **2.3.8 Test for gum and mucilages ( Whistler and Bemiller, 1993 )**

About 5 ml of all the extract was slowly added to 5 ml of absolute alcohol under constant stirring. The test was performed individually for all the extracts individually. Presence of gum and mucilages were identified through the occurrence of precipitates.

### **2.3.9 Test for tannins( Trease and Evans, 1989 )**

To 1 ml of each of the solvent extract, a few drops of  $\text{FeCl}_3$  solution was added. The appearance of black and a green precipitate indicated the presence of tannins.

### **2.3.10 Acetone – water test for resins**

The extracts were treated with acetone followed by a small amount of water and shaken well. Appearance of turbidity indicated the presence of resins .

### **2.3.11 Test for terpenoids (Evans, 1997)**

To 1 ml of each of the solvent extract, 2 ml of chloroform was added. Then 3 ml of concentrated  $\text{H}_2\text{SO}_4$  was carefully added to form a layer. A reddish brown coloration of the interface showed the presence of terpenoids.

### **2.3.12 Ferric chloride test for phenols(Mace, 1963)**

To 1 ml of solvent extract 3 ml of distilled water was added. To this, a few drops of neutral 5%  $\text{FeCl}_3$  solution was added. Presence of phenols were confirmed through the formation of a dark green colour.

### **2.3.13 Foam test for saponins (Kumar *et al.*, 2009)**

About 2 ml of distilled water and 1 ml of each solvent extract were mixed and shaken vigorously. Formation of a stable persistent froth indicated the presence of saponins.

### **2.3.14 Keller – killani test for cardiac glycosides(Sofowora, 1984)**

The extract was dissolved in glacial acetic acid containing traces of  $\text{FeCl}_3$ . The tube was then held at an angle of  $45^\circ \text{C}$  and 1 ml of concentrated  $\text{H}_2\text{SO}_4$  was added along the

sides of the tubes. Formation of a purple ring at the interphase indicated the presence of cardiac glycosides.

#### **2.3.15 Test for volatile oils**(Trease and Evans, 1989 )

To 1 ml of each extract, 1 ml of 90% ethanol was added followed by the addition of few drops of  $\text{FeCl}_3$  solution . Formation of green colour would indicate the presence of volatile oils .

#### **2.3.16 Test for starch**

The plant extracts ( 5 ml ) were treated with few drops of iodine reagent to get a blue violet colour. Apperence of the same revealed the presence of starch.

#### **2.3.17 Test for betacyanins**(Harbone, 1973)

With 2 ml of each of the plant extract, 1 ml of 2N NaOH was added and heated for 5 minutes in water bath. Formation of yellow color indicated the presence of betacyanin .

#### **2.3.18 Test for coumarins**

About 3 ml of 10% Sodium hydroxide ( NaOH ) solution was added to 2 ml of plant extracts individually. Formation of yellow color indicated the presence of coumarins.

#### **2.3.19 Test for steroids** (Kokate, 1994)

Plant extracts (1ml) were dissolved in 10 ml of chloroform and then equal volume of concentrated sulphuric acid was added from the side of test tubes. red Upper layer turned red and sulphuric acid layer showed yellow with green fluorescence. This result suggested the presence of steroids.

#### **2.3.20 Test for anthraquinones**(Sofowora, 1993)

Plant extracts ( 0.5 ml ) were boiled with 10% HCl for few minutes on water bath. The reaction mixture was filtered and allowed to cool. Equal volume of chloroform was added to each filtrate and few drops of 10% ammonia was added and then heated. Formation of rose to pink color was observed.

### **2.3.21 Test for phlobatannins**(Harbone, 1973)

2 ml of all the plant extracts were boiled individually with 1 ml of 1% aqueous hydrochloric acid. The formation of red precipitate indicated the presence of phlobatannins.

### **2.3.22 Test for emodols**

With 2 ml of plant extracts, 2 ml of ammonium hydroxide (NH<sub>4</sub>OH) and 3 ml of benzene was added. Appearance of red color indicated the presence of emodins.

### **2.3.23 Test for fatty acids**(Ayoola *et al.*, 2008)

About 2ml of extract was treated with few drops of Sudan III reagents. The appearance of dark red oil droplet in the upper layer indicated the presence of fatty acids.

## **3. RESULTS AND DISCUSSION**

Plants used in traditional medicine contain a lot of bioactive materials which play a major role in treating infectious diseases. (Balakumaret *al.*, 2011; Florence *et al.*, 2012; Joselin *et al.*, 2012; Rajan *et al.*, 2011). Natural biologically active compounds of plants are classified into major groups such as sterols, triterpenes, flavones, emodols, coumarins, tannins, reducing compounds, glycosides, saponins, sapogenins etc. All these substances contribute to the medicinal properties of plants. Basically *Artocarpus* species consists of phenolic compounds, flavonoids, jacalin, lectin and stilbenoids. *Artocarpus* extracts and metabolites from leaves, stem, fruit and bark contain numerous beneficial biologically active compounds and these compounds are used for various biological activities including antibacterial, antitubercular, antiviral, antifungal, antiplatelet, antiarthritic, tyrosinase inhibitory and cytotoxicity (Jagtap & Bapat, 2010). Among the 23 phytochemicals tested using five different cold percolation extracts of *Artocarpus altalis* unripe fruit, carbohydrates, quinones, terpenoids, cardiac glycosides and starch showed presence in all the 5 extracts analysed. Quinones and cardiac glycosides were weakly present in hexane extract while starch showed moderate presence. Moderate and weak presence of terpenoids and cardiac glycosides were seen in aqueous, methanol and acetone extracts while in ethyl acetate and hexane these metabolites could not be detected. Suvarna *et al.* (2014) reported the presence of flavonoids, saponins, terpenoids etc in different organic solvent extracts of *Artocarpus hirsutus* edible fruit part. Aqueous extract of unripe fruits showed strong intensity of amino acids,

flavonoids, gums and mucilages, resins, phenols and cardiac glycosides while tannins and saponins were moderately present. Proteins and reducing sugars could not be traced in aqueous extract of the fruit sample.

Steroids showed weak presence in all the extracts of unripe fruit. Strong presence of amino acids was also noted in the methanolic extract of fruits. Moderate and weak presence of amino acids were exhibited by ethyl acetate and acetone fraction of fruits. Hexane extract of unripe fruits did not show the presence of amino acids (Table 1).

Mohanty and Pradhan (2014) screened the petroleum ether, methanol and ethyl acetate extracts of breadfruit and reported the presence of steroids, phenols and flavonoids in methanolic fraction of fruit. In the present investigation proteins exhibited high intensity in the acetone and methanolic unripe fruit extracts, followed by weak intensity in ethyl acetate fraction however this biomolecule could not be traced in the aqueous and hexane extracts. Golden and Williams (2001) identified an increase in the amino acid, carbohydrate and fatty acid content of *Artocarpus altissima* when the unripe green fruits changed to yellow ripe ones. Reducing sugars could not be detected in hexane extracts of unripe fruits, but it showed weak presence in ethyl acetate extract, moderate presence in acetone extract and strong presence in methanol extract (Table 1).

Flavonoid screening in the ethyl acetate and hexane unripe fruit extract failed to show its presence, while in the acetone and methanol extracts its weak presence was observed. Aqueous extract of unripe fruits showed strong occurrence of flavonoids (Table 1). Gums and mucilage could not be detected in the hexane extract and ethyl acetate extract of unripe fruits and its weak presence was noted in acetone extract of fruit while methanol fraction of unripe fruits was found to possess high level of gums and mucilage (Table 1). Phytochemicals like phenols and tannins were seen in high intensity in the methanol extract of unripe fruits, while, ethyl acetate, hexane and acetone extracts these phytochemicals could not be detected.

Resins were strongly detected in acetone and methanol extract of unripe fruits while it was weakly found in aqueous extract. Ethyl acetate and hexane fractions reported complete absence of resins. The most important factor to be highlighted is the strong presence of terpenoids in all the 5 fruit extracts. Cardiac glycosides were strongly detected in all the fruit extracts except hexane which exhibited its weak presence.

Volatile oil was also strongly present in the ethyl acetate, acetone and methanolic extract of

breadfruit while aqueous and hexane extracts did not show its presence. Total absence of alkaloids and phlobatannins were reported in all the five extracts of fruit. Jiyauddin *et al.* (2014) screened the methanolic extract of breadfruit for the presence of various phytochemicals and reported the presence of phenolics, flavonoids, glycosides, steroids and absence of alkaloids, tannins, and saponins. These results could be correlated with the present findings except for the absence of tannins.

Coumarins and emodols were weakly detected in the methanol extract of unripe fruits while betacyanins were seen in the acetone extract, while this compound could not be detected in the other extracts. Strong presence of anthraquinones and fatty acids were seen in the methanolic extract of unripe fruits (Table 1).

The presence of compounds such as acids, quinones, tannins, gums and mucilages, phenols, steroids, saponins, flavonoids, terpenoids, anthraquinones, betacyanin, resins, volatile oils, cardiac glycosides, fixed oils and fats, coumarins, reducing sugar and emodols in different cold percolation solvent extracts of ripe fruits of *Artocarpus heterophyllus* were earlier reported revealing methanol as the best solvent of choice to elute maximum number of phytochemicals from their respective fruit sample belonging to *Moraceae* family. (Jaya *et al.*, 2018). Overall results of the present study also suggested methanol as the better solvent of choice among other solvents used to elute maximum phytochemicals from unripe fruit of *Artocarpus alticola*. This capability of methanol to extract maximum phytochemicals may be attributed to its amphiphilic nature. Methanol is an amphiphilic solvent with a polar water – soluble group attached to a water – insoluble hydrocarbon chain (Primorac *et al.*, 2018). Also breadfruit might be rich in phytochemicals with better solubility in methanol than in rest four solvents used for extraction.

All the plant metabolites identified here accounts for varied medicinal properties of plants. *Artocarpus* contains rich prenylated phenolic compounds such as geranylated flavones (Badrie and Broomes, 2010). Flavonoids are effective antioxidants and show stronger anticancer activities (Salah *et al.*, 1995; Del Rio *et al.*, 1997; Okwu, 2004). Carbohydrate based or modified therapeutics are used extensively in cardiovascular and hematological treatments ranging from inflammatory diseases and anti-thrombotic treatments to wound healing. (Kilcoyne & Joshi, 2007). Quinones, by their antioxidant activity improve the general health conditions. Many clinically approved drugs are quinine related compounds. (Najjar *et al.*, 2011). Varatharajan *et al.* (2012) proved that terpenoids increase blood flow to the brain improve memory in people with mild dementia and also used to treat



cancer and inflammatory diseases. According to many reports, glycosides lower blood pressure (Nyarko and Addy, 1990). Medicinal, pharmaceutical and agrochemical activities like antitumour, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, antihelminthic, cytotoxic and cardiogenic activity of plant steroids were reported by Patel and Savjani (2015). Research on marketing of drugs derived from resins point to the possibility of their further medical use in the west as well as in the countries where traditional use of resins happened. (Singh *et al.*, 1993). Potential usage of gum and mucilages in various pharmaceutical preparations for novel drug delivery system (NDDS) was accomplished by Reddy and Koppam (2013). Irshad (2009) reported the antioxidant, antibacterial, antiviral, anticancer and insecticidal activity of volatile oil as well as its role in food preservation and aromatherapy. Betacyanins possess antioxidant properties beneficial in the prevention of cancer and cardiovascular diseases (Leong *et al.*, 2017). Similarly, inhibition of cancer growth through induction of apoptosis by anthraquinones was reported by Khan (2019). Unsaturated fatty acids with potential therapeutic activities were identified in *Artocarpus altii* soil by Aremuet *et al.* (2017). Breadfruit also includes Artocarpins, phenolic compounds and lectins associated with anti-inflammatory activity (Watson and Preedy, 2009). Reports indicated that a high percentage of breadfruit carbohydrate was present mostly in the form of starch. Powdery form of breadfruit starch could be used as an excipient in paracetamol tablet formulation at 5% w/v concentration (Ross, 2009).

**TABLE 1: PHYTOCHEMICAL ANALYSIS OF UNRIPE FRUIT EXTRACTS OF *ARTOCARPUS ALTIIS* (PARKINSON) FOSBERG**

S.No.	Compounds	Aqueous	Ethyl Acetate	Hexane	Acetone	Methanol
1.	Carbohydrates	+++	+++	+++	+++	+++
2.	Quinones	+++	+++	+	+++	+++
3.	Alkaloids	-	-	-	-	-
4.	Aminoacids	+++	++	-	+	+++
5.	Proteins	-	+	-	+++	+++
6.	Reducing sugars	-	+	-	++	+++
7.	Flavonoids	+++	-	-	+	+
8.	Gums & Mucilages	+++	-	-	+	+++
9.	Tannins	++	-	-	-	+++
10.	Resins	+++	-	-	+++	+++
11.	Terpenoids	+++	+++	+++	+++	+++
12.	Phenols	+++	-	-	-	+++

13.	Saponins	++	-	-	-	-
14.	Cardiac glycosides	+++	+++	+	+++	+++
15.	Volatile oils	-	+++	-	+++	+++
16.	Starch	++++	+++	++	+++	+++
17.	Betacyanins	-	-	-	+	-
18.	Coumarins	-	-	-	-	+
19.	Steroids	+	+	+	+	+
20.	Anthroquinones	-	-	-	-	+++
21.	Phlobatannins	-	-	-	-	-
22.	Emodols	-	-	-	-	+
23.	Fatty acids	-	-	-	-	+++

++++ abundantly present; +++strongly present; ++ moderately present; + weakly present; -absent

### 3 CONCLUSION

Several research works had been conducted on *Artocarpusaltilis* in order to reveal its phytochemical properties by many researchers from different parts of the world. The present work represents an elaborate comparative phytochemical screening of mature unripe fruit of *Artocarpusaltilis*. The investigation revealed that unripe fruits of *Artocarpusaltilis* is a good source of phytoconstituents. Present phytochemical findings are in agreement with many of the reported literatures in the *Moraceae* family. Separation and purification of important metabolites and subsequent structural studies will open the way for obtaining active compounds from this plant and therefore provides scope for scientific studies in future to fight with emerging diseases. Moreover the position of this fruit (both unripe and ripe) in the culinary dishes of Asia holds more importance in the time of many pandemics in the past and in the present (in the age of Covid 19). With its easy accessibility to the commoner, this fruit is going to be a food for all in the near future as food and may be break through medicines.

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